KIDNEY FUNCTION, BONE-MINERAL METABOLISM MARKERS, AND FUTURE RISK OF PERIPHERAL ARTERY DISEASE: THE ATHEROSCLEROSIS RISK IN COMMUNITES (ARIC) STUDY

by

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ABSTRACT

Background: Kidney dysfunction is a known risk factor for lower-extremity peripheral artery disease (PAD). Although novel filtration markers like cystatin C and beta-2-microglobulin demonstrate stronger associations with coronary heart disease, stroke, and heart failure compared to creatinine-based estimated glomerular filtration rate (eGFRcr), whether this pattern holds for PAD is unclear. Also, bone-mineral disorders are important complications of kidney dysfunction, but little is known whether bone-mineral metabolism (BMM) markers are associated with future PAD risk beyond kidney function.

Methods: Using data from 12,437 ARIC Study participants free of clinical history of PAD at baseline (1990-1992), we first quantified the associations of clinical categories of eGFR based on creatinine, cystatin C, and both with PAD risk. Subsequently, we evaluated quartiles of these eGFRs, cystatin C, beta-2 microglobulin, as well as BMM markers (fibroblast growth factor 23, parathyroid hormone, calcium and phosphorus). PAD was defined as hospitalizations with ICD-9 codes for lower-extremity atherosclerosis, revascularization and amputation.

Results: During a median follow-up of 21 years, 471 participants had at least one hospitalization with a discharge code for PAD. Low eGFR was significantly associated with future PAD risk, independent of traditional cardiovascular risk factors, with slightly stronger relationship when cystatin C was used (5.3-7.5 fold higher risk for eGFR <30 and 2.5-3.7 fold higher risk for eGFR 30-59 vs. eGFR ≥90 ml/min/1.73m^2). Novel filtration markers, particularly B2M, appeared to have stronger association with incident PAD than eGFRcr (adjusted hazard ratios (HRs) for top vs. bottom quartile 2.85 (95% CI: 2.10- 3.88) vs. 1.30 (95% CI: 0.98-1.71)). The association was consistent after

adjustment for BMM markers or restricting to critical limb ischemia as an outcome. Among BMM markers, top vs. bottom quartile of phosphorus remained significant for PAD risk beyond potential confounders including kidney function (HR 1.43, 95% CI: 1.08-1.88).

Conclusions: Kidney dysfunction was significantly associated with future PAD risk independently of potential confounders and BMM markers, particularly when cystatin C and B2M were taken into account. Among BMM markers, phosphorus was most robustly associated with future PAD. Our results suggest the usefulness of novel filtration markers for PAD risk assessment and the unique contribution of phosphorus to the pathophysiology of PAD.

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PREFACE AND ACKNOWLEDGEMENTS

In August 2014, I started to pursue the Master of Science degree in epidemiology with concentration in cardiovascular disease in the Department of Epidemiology at Johns Hopkins University Bloomberg School of Public Health in Baltimore, Maryland, after I completed the Bachelor of Medicine degree from Fudan University Shanghai Medical College in July that year in Shanghai, China.

To fulfill the requirements for a Master of Science degree in epidemiology, I began working as a research analyst, advised by Dr. Kunihiro Matsushita, on my thesis studying the associations between peripheral artery disease and kidney function markers using data from an ongoing community-based cohort study, the Atherosclerosis Risk in Communities (ARIC) study since July 2015, after having completed the required courses for the degree in the first year.

This study was approved by Johns Hopkins Bloomberg School of Public Health Institutional Review Board Office (IRB No: H.34.99.07.02.A1). As a student investigator on the study team, I performed data analysis and composed the manuscript, under the supervision of my advisor, Dr. Matsushita.

This thesis is original, unpublished and independent work by the author, Chao Yang.

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BACKGROUND AND HYPOTHESES

Peripheral artery disease (PAD), characterized by atherosclerosis of the lower extremities, [1] affects more than 8 million adults in the US, increases the risk of adverse health outcomes, [2] and impacts functional performance. [3] PAD is especially an important complication for those with chronic kidney disease (CKD), particularly at more advanced stages. [4-6] Indeed, the incidence of PAD is higher than that of myocardial infarction and stroke among dialysis patients. [7] Of note, mildly to moderately reduced kidney function has also been associated with higher risk of PAD in several reports. [8-10]

Since those reports were published, new equations for estimated glomerular filtration rate (eGFR) (e.g., the CKD-EPI equations) or novel filtration markers (e.g., cystatin C and β2-microglobulin (B2M)) have demonstrated stronger associations with cardiovascular events as compared to the more traditional measure of kidney function, creatinine-based eGFR using the MDRD Study equation. [11-16] However, to our knowledge, those new equations and novel filtration markers have not been exclusively used to assess the risk of incident PAD.

Moreover, patients with kidney dysfunction are prone to have abnormal bone-mineral metabolism, with altered levels of fibroblast growth factor 23 (FGF-23), parathyroid hormone (PTH), serum calcium, and serum phosphorus.[17-20] These bone-mineral metabolism (BMM) biomarkers are reported to partially explain excess cardiovascular risk among persons with CKD [17 21-24] but have not been comprehensively evaluated in the context of PAD risk.

Therefore, we comprehensively assessed the association of future risk of PAD with multiple measures of kidney function and BMM using data from a bi-racial community-based cohort, the Atherosclerosis Risk in Communities (ARIC) study. Our key hypotheses are as follows: 1) novel filtration markers are more strongly associated with PAD risk compared with creatinine-based eGFR; 2) BMM markers attenuate the association between kidney function and PAD risk; 3) and BMM markers are associated with future PAD risk beyond kidney function.

METHODS

Study design and population

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective cohort of 15,792 individuals aged from 45 to 64 years at visit 1 (1987-1989) from four communities in the US (Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, MD). The ARIC Study has conducted follow-up visit 2 (1990-1992), visit 3 (1993- 1995), visit 4 (1996-1998) and visit 5 (2011- 2013). We used ARIC Study data from visit 2, when all measures of kidney function and BMM markers of interest were collected, as baseline in this study. Of the 14,348 participants who attended visit 2, we excluded participants with race other than black or white (n= 42), missing data on variables of interest (n= 1,832), or with a clinical history of PAD at baseline determined by self-report leg artery revascularization at visit 1 and any PAD-related hospitalizations prior to the visit of interest at visit 2 (n= 37), yielding a final sample of 12,437 participants. Written informed consent was obtained from all participants.

Measurements

For this study, participants' baseline demographic, life-style, and medical characteristics were collected at visit 2, if not otherwise specified. Smoking status and alcohol drinking status were self-reported and categorized into current, former or never. Education information was obtained at visit 1 and was categorized into 3 groups: basic (less than high school), intermediate (high school graduate or vocational school), and advanced (college, graduate school, or professional school). Body mass index (BMI) was calculated as weight in kilogram divided by height in meter squared. Sitting blood pressures were measured thrice after a 5-minute rest using a sphygmomanometer, and the average of the last two was recorded. Diabetes was diagnosed as fasting plasma glucose level ≥126 mg/dL (≥7.0 mmol/L), non-fasting glucose level ≥200 mg/dL (≥11.1 mmol/L), self-reported physician diagnosis of diabetes, or use of anti-diabetic medications. Prevalent coronary heart disease was defined as cases adjudicated by physician-panel between visits 1 and 2 in addition to self-reported clinical history and evidence of prior myocardial infarction by electrocardiogram obtained at visit 1. Prevalent stroke was similarly defined by self-reported history at visit 1 and any adjudicated cases between visits 1 and 2. Medications were determined via self-report usage in the past 2 weeks. All serum samples were obtained from participants who were asked to fast for 12 hours before their visit and stored at -80 ℃ according to standardized protocols. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured using automated enzymatic procedures, [25 26] and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[27]

Kidney filtration and bone-mineral metabolism markers

eGFR was calculated by the CKD-EPI (CKD Epidemiology Collaboration) [28 - 30] equations based on demographical variables such as age, sex and race and either or both filtration markers, serum creatinine and cystatin C (i.e., eGFRcr, eGFRcys, and eGFRcr-cys, respectively) . eGFRcr-cys was chosen as the primary kidney filtration marker because it is the best available estimate for measured GFR.[31] Serum creatinine was measured by a modified kinetic Jaffé method, with reliability coefficient of 0.95. Serum cystatin C and B2M were measured by a particle-enhanced immunenephelometric assay using a BNII nephelometer (Siemens Healthcare Diagnostics), with reliability coefficient 0.94 for cystatin C and 0.98 for B2M.[16]

FGF-23 was measured in singlicate via a 2-site ELISA (FGF-23 ELISA Kit; Kainos Laboratories, Tokyo, Japan) at the Advanced Research and Diagnostic Laboratory, University of Minnesota (Minneapolis, MN) in serum samples collected during ARIC visit 2, with coefficient of variation (CV) 16.6% from split paired samples and 8.8% from internal laboratory quality control samples at 41.4 pg/mL.[32] PTH was measured using a sandwich immunoassay method on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation), with CV 9.7%. Serum calcium and phosphorus were measured using colorimetric methods on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation), with CV 2.4% and 3.0% for calcium and phosphorus, respectively. [33]

Definition of peripheral artery disease (PAD)

Based on previous literature,[10 34] clinical PAD was identified according to hospitalizations with the following ICD-9 discharge codes: 440.20 (atherosclerosis of

native arteries of the extremities, unspecified); 440.21 (atherosclerosis of native arteries of the extremities with intermittent claudication); 440.22 (atherosclerosis of native arteries of the extremities with rest pain); 440.23 (atherosclerosis of native arteries of the extremities with ulceration); 440.24 (atherosclerosis of native arteries of the extremities with gangrene); 440.29 (other atherosclerosis of native arteries of the extremities); 440.3 (atherosclerosis of bypass graft of the extremities); 440.4 (Chronic total occlusion of artery of the extremities), 38.18 (endarterectomy, lower limb arteries), 39.25 (intravascular imaging of non-coronary vessel(s) by optical coherence tomography), 39.29 (other (peripheral) vascular shunt or bypass), 39.50 (angioplasty or atherectomy of other non-coronary vessel(s)). Of PAD cases, participants with 440.22 (atherosclerosis of native arteries of the extremities with rest pain); 440.23 (atherosclerosis of native arteries of the extremities with ulceration); 440.24 (atherosclerosis of native arteries of the extremities with gangrene) and those with any of the code above with concurrent ICD-9 codes of ulcer (707.1), gangrene (785.4), and leg amputation (84.1x) were considered as critical limb ischemia (CLI), the most severe form of PAD, with substantial impact on patients' prognosis and quality of life as well as medical expenditure.[35-37] Follow-up of participants who were free of PAD ended on the date of death, date of last contact, or December 31, 2012, whichever came first.

Statistical analysis

We compared participants' baseline characteristics by incident PAD status using two sample t test, Wilcoxon rank sum test, and chi square test, as appropriate. Spearman rank

correlation coefficient was calculated between kidney filtration markers and BMM markers.

For longitudinal analyses of incident PAD, we first visualized the potential dose-response relationship between eGFRs and PAD. We estimated incidence rate according to eGFRs as linear splines with six knots at 30, 45, 60, 75, 90 and 105 ml/min/1.73m^2, adjusting for age, gender and race, in Poisson regression models. Subsequently, we examined the impact of potential confounders (details described below) using Cox proportional hazards regression models across clinically meaningful categories of eGFRs, ≥90, 60-<90, 30-<60, and <30 mL/min/1.73 m^2.[38] Then, for a fair comparison of each of the filtration markers, we investigated their quartiles (top quartile as reference for all three eGFRs, and the lowest quartile as reference for the rest of markers). BMM markers were similarly modeled as quartiles.

To acknowledge the attenuation of the associations between filtration markers and PAD risk by accounting for BMM markers, we built two models: Model 1 adjusted for age, gender, race and ARIC visit center, and traditional confounders at baseline including education level, BMI, smoking status, alcohol drinking status, LDL cholesterol level, HDL cholesterol level, systolic blood pressure, use of anti-hypertensive medications, use of cholesterol-lowering medications, diabetes mellitus, prevalent coronary heart disease and prevalent stroke; and Model 2 additionally adjusted for all four BMM markers. For the analysis of BMM markers as key exposures, we built three models (denoted as Model I to III) to test whether BMM markers are associated with future PAD risk and CLI after adjusting for demographics, traditional cardiovascular risk factors, and kidney function. Specifically, Model I adjusted for basic demographic confounders including age, gender,

race and ARIC visit center, Model II additionally adjusted for traditional cardiovascular risk factors (education level, BMI, smoking and alcohol drinking status, LDL cholesterol level, HDL cholesterol level, systolic blood pressure, use of anti-hypertensive medication, use of cholesterol-lowering medication, diabetes mellitus, prevalent coronary heart disease and prevalent stroke), and Model III further adjusted for eGFRcr-cys.

Finally, we conducted subgroup analysis by key demographic and clinical subgroups according to age (<65 vs. ≥65 years), gender, race (white vs. black), smoking status (current/ former vs. never) and the presence/absence of diabetes mellitus, hypertension (defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure≥ 90 mmHg, or taking any anti-hypertensive medication), cardiovascular disease (defined as with either prevalent coronary heart disease or stroke) at baseline. Potential effect measure modification was tested using likelihood ratio test by comparing models with and without interaction terms.

All analyses were performed using Stata, version 13.0 (StataCorp LP, College Station, Texas), and statistical significance level was set at 0.05 for all hypothesis testings.

RESULTS

Baseline characteristics

The average age of the participants was 56.9 years old (SD 5.7 years), and 56.5% (n=7,022) were females, and 75.5% (n=9,388) were whites. As shown in Table 1, compared with participants who did not develop incident PAD during the follow-up, those who developed PAD were more likely to be older, men, blacks, current smokers,

have diabetes and history of stroke and coronary heart disease, higher BMI, SBP, LDL cholesterol level and lower HDL cholesterol level, and take cholesterol-lowering medication and anti-hypertensive medication, while less likely to be highly educated or current drinkers. As shown in Table S1, kidney filtration markers were moderately to highly correlated to each other (Spearman rank correlation coefficient (|r|) ranges from 0.46 to 0.97) but weakly to BMM markers ((|r| ranges from 0.01 to 0.24) (Table S1). Correlations between BMM markers were also weak (|r| ranges from 0.08 to 0.20).

Associations of eGFRs with incident PAD and CLI

Out of 12,437 participants who were free of PAD at baseline, 471 participants had at least one hospitalization with a discharge code for PAD (crude incidence rate: 2.07 cases/1,000 person-years) during a median follow-up of 21 years, and 171 participants had at least one hospitalization with a discharge code for CLI (crude incidence rate: 0.74 cases/ 1,000 person-years) during a median follow-up of 21 years. Figure 1 shows demographically-adjusted incidence rate of PAD according to eGFRs. Regardless of equations used, incidence rate of PAD increased steadily below eGFR 105 mL/min/1.73 m^2, with risk gradient of 6-8 fold between eGFR 15 and 105 mL/min/1.73 m^2. As seen in other cardiovascular outcomes [39], increased incidence rate of PAD was also observed at high eGFR, when creatinine was used for estimating GFR (Figure 1A and 1C). This pattern was not necessarily evident for eGFRcys (Figure 1B).

With clinical categories of eGFR, we confirmed that the associations with PAD risk remained significant with a stepwise increased risk with decreasing eGFR categories even after accounting for other traditional cardiovascular risk factors (Model 1 in Table

2). Specifically, participants with eGFRs <30 mL/min/1.73 m^2 had more than 10-fold higher risk of incident PAD compared to those in the reference group with eGFR ≥90 mL/min/1.73 m^2. eGFR 30-59 mL/min/1.73 m^2 contributed to 2.5-3.8 folds higher risk compared to the reference group. Of note, even those with mildly reduced eGFR 60-89 mL/min/1.73 m^2 demonstrated 1.2-1.5 folds higher risk of PAD compared to the reference. The hazard ratios (HRs) of PAD in each eGFR category were highest for eGFRcr-cys. When we adjusted for all four BMM markers, the attenuation was evident only in the category of eGFR <30 mL/min/1.73 m^2 (Model 2 in Table 2), but the associations still remained significant. The attenuation was mainly driven by PTH (Table S2). We observed similar associations when CLI was investigated as the outcome of interest (Table 2).

According to the association between eGFRs and incident PAD observed in Figure 1 and Table 2, to obtain reliable estimates in every subgroup, we assessed whether the HR of PAD for every 15-unit lower eGFR below 105 ml/min/1.73m^2 would differ in subgroups by age, sex, race, ever/never smoking, hypertension, diabetes and prevalent cardiovascular disease status while adjusting for traditional cardiovascular risk factors plus BMM markers (i.e., Model 2) (Table S3). We found consistent association between low eGFR and incident PAD in all subgroups. We found significant interaction by ever smoking status, diabetes status and hypertension regardless of eGFR equations, but importantly lower eGFR was positively associated with PAD risk in both strata of these subgroups.

Associations of novel filtration markers with incident PAD and CLI

When we contrasted novel filtration markers, cystatin C and B2M with eGFRs using their quartiles (Table 3), overall, B2M appeared to be most strongly and consistently associated with future PAD risk, with 2.5-2.8 fold higher risk between Q4 vs. Q1. Q3 showed significant results only for B2M, and even its Q2 reached significance for CLI. As the PAD risk was lowest in Q2 for eGFRs in several models, we also compared the associations with Q2 as reference and confirmed the highest HRs for Q4 of B2M (Table S4). We confirmed similar associations for quartiles of cystatin C and B2M across the subgroups tested (Table S5).

Associations of BMM markers with incident PAD and CLI

Neither of PTH or calcium demonstrated significant associations with PAD risk in all Models I to III with Q1 as reference (Table 4). FGF23 was significantly associated with incident PAD when adjusted for traditional cardiovascular confounders (Q4 vs. Q1 HR= 1.41, 95% CI: 1.09 to 1.82 in Model II); however, the association was no longer significant after further adjusting for eGFRcr-cys (Model III). Phosphorus was the only BMM marker showing significant association with incident PAD, independently of traditional cardiovascular risk factors as well as kidney function. The HR of incident PAD comparing Q4 to Q1 for phosphorus was 1.52 (95% CI: 1.15 to 2.01) in Model II, and 1.43 (95% CI: 1.08 to 1.88) in Model III. Similar patterns were observed for CLI as the outcome.

DISCUSSION

In this large community-based cohort study, we observed that reduced eGFR, regardless of equations, was associated with future PAD risk in the general population, independently of known traditional cardiovascular risk factors. Participants with baseline eGFR <30 ml/min/1.73m^2 had over 10-fold higher risk of PAD compared to those with eGFR ≥90 ml/min/1.73m^2. Although weaker, eGFR 30-<60 and 60-<90 still conferred a ~3-fold and a ~1.5-fold higher risk of future PAD, respectively. Of note, the association tended to be more obvious when kidney function was assessed with novel filtration markers such as cystatin C and B2M compared to a more conventional measure used in clinical practice, eGFRcr. The associations were generally consistent across various demographic and clinical subgroups. The adjustment for BMM markers attenuated the associations between kidney function and PAD risk solely when eGFR was below 30 ml/min/1.73m^2. Overall BMM markers were not consistently associated with PAD risk even in demographically adjusted models; however, phosphorus was the only marker showing significant and consistent associations after accounting for traditional cardiovascular risk factors and kidney function. Based on a large sample size and long follow-up time, we confirmed similar patterns for CLI, a more severe form of PAD.

Several studies have reported the association between kidney function and PAD. [8 10 40-45] However, ours is one of a few studies exploring this association in a prospective design. [8-10] Using state-of-the-art equations for eGFR and based on adequate PAD cases over long follow-up, we were able to quantify the dose-response relationship across clinical categories of different eGFR equations. Further, the analysis specific to CLI seems unique. Taken altogether, our results clearly support the important contributions of

reduced kidney function to the pathophysiology of PAD development. FGF23, PTH, and phosphorus are considered as potential mechanisms linking CKD to systemic atherosclerosis [22-24 46-48 49], and our results suggest these BMM markers may partially explain this association between CKD and PAD risk when kidney function is severely reduced. Other suggested mechanisms linking CKD to atherosclerotic disease include inflammation, activated coagulation system, altered homocysteine metabolism, and oxidative stress. [50-53]

Similar to other subtypes of cardiovascular outcomes, we observed that novel filtration markers, cystatin C and B2M, are more strongly associated with PAD compared to creatinine-based eGFR.[16] As discussed previously, there may be kidney-related and non-kidney elements behind this observation. [54] Specifically, cystatin C and B2M may be superior to serum creatinine as filtration markers and indeed are less affected by diet and muscle mass.[31] In terms of non-kidney elements, cystatin C and B2M are known to be linked to inflammation, which may contribute to their strong associations with atherosclerotic disease. In addition, B2M has been shown to be indicative of amyloid deposition and aggregation in the vessel wall that may contribute to atherosclerosis process.[55 56] Of note, in a proteomic profiling study, out of ~1,600 protein peaks assessed, B2M was found to be most robustly associated with PAD.[57]

We did not observe consistent associations of multiple BMM markers with PAD risk. The results for PTH are not necessarily surprising as it was not strongly associated with several cardiovascular diseases in a previous report from the ARIC Study.[33] FGF-23 has been shown to be associated with other subtypes of cardiovascular disease in some studies, [58 59] but its association with PAD in our study was no longer significant once

adjusting for kidney function. Of those BMM markers, phosphorus was the only significant one associated with PAD even after accounting for kidney function. Although we are not completely sure about the mechanisms behind this link, our findings are in line with a previous study showing the relationship between phosphorus and the risk of composite cardiovascular outcomes including coronary heart disease, stroke, PAD, and heart failure. [49] Unfortunately, that previous study did not report specific results for PAD. Serum phosphorus is known to induce vascular calcification through mineralization of the extracellular matrix [60]. High phosphorus levels would also inhibit 1,25- dihydroxy vitamin D synthesis [24 61], which might subsequently increase vascular calcification. Nonetheless, future investigations of the mechanisms linking phosphorus to the development of PAD are warranted.

Our study results may have some clinical and public health implications. These results highlight that reduced kidney function, even at mild to moderate stage, is a risk factor for PAD and CLI, independently of well-known traditional cardiovascular risk factors. Besides, novel filtration markers may lead to better risk stratification of future PAD. Given a possible role for phosphorus in the pathophysiology of PAD suggested in our study, future investigations would be warranted regarding whether controlling serum phosphorus levels could result in reduction of PAD risk. This may be important since there are no established treatments to recover kidney function, but phosphate-binding agents are clinically available. In this context, it may be important to recognize that high phosphorus diet also contributes significantly to serum phosphorus levels.[62]

Admittedly, this study had several limitations. First, identifying PAD and CLI cases based on ICD-9 from hospitalization records limited the sensitivity in outcome

ascertainment, though this method is likely to be specific and capture severe symptomatic patients requiring hospitalizations. Secondly, kidney function and BMM markers as well as other covariates were measured only once at baseline, which may lead to potential misclassification. However, this type of misclassification usually results in more conservative estimates. Finally, we cannot rule out the possibility of residual confounding, as true in any observational studies.

In summary, reduced kidney function, even at mild to moderate stages, is independently associated with future PAD risk. The associations are more evident when novel filtration markers, cystatin C and B2M, are assessed. Although overall the associations between BMM markers and PAD risk were not consistent, serum phosphorus demonstrates a robust association. These findings confirm the importance of PAD as a complication in persons with reduced kidney function. Also, our findings suggest usefulness of novel filtration markers for assessing PAD risk and a potential role of phosphorus in the pathophysiology of PAD.

TABLES

Table 1 Baseline characteristics of participants by incident PAD status

Variables	Total	Incident PAD		p-value
	(n=12437)	Yes (n=471)	No (n=11966)	
Age (years)	56.9 ± 5.7	58.8 ± 5.6	56.8 ± 5.7	<0.001
Male	5415 (43.5)	258 (54.8)	5157 (43.1)	<0.001
Blacks	3049 (24.5)	142 (30.1)	2907 (24.3)	0.004
Education				
Basic	2614 (21.0)	159 (33.8)	2455 (20.5)	<0.001
Intermediate	5203 (41.8)	177 (37.6)	5026 (42.0)	
Advanced	4620 (37.2)	135 (28.7)	4485 (37.5)	
Body mass index (kg/m^2)	28.0 ± 5.4	29.0 ± 5.8	27.9 ± 5.4	<0.001
Average systolic blood pressure (mmHg)	121.2 ± 18.6	128.8 ± 20.9	120.9 ± 18.4	<0.001
Average diastolic blood pressure (mmHg)	72.1 ± 10.2	72.0 ± 11.2	72.1 ± 10.2	0.943
Use of anti-hypertensive medication	3281 (26.4)	212 (45.0)	3069 (25.6)	<0.001
LDL cholesterol (mg/dL)	133.4 ± 36.7	142.7 ± 40.2	133.1 ± 36.5	<0.001
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.1 ± 0.4	1.3 ± 0.4	<0.001
Use of cholesterol-lowering medication	777 (6.2)	56 (11.9)	721 (6.0)	<0.001
Smoking				<0.001
Current	2723 (21.9)	177 (37.6)	2546 (21.3)	
Former	4698 (37.8)	174 (36.9)	4524 (37.8)	
Alcohol drinking				0.006
Current	7058 (56.8)	250 (53.1)	6808 (56.9)	
Former	2574 (20.7)	125 (26.5)	2449 (20.5)	
Prevalent coronary heart disease	683 (5.5)	92 (19.5)	591 (4.9)	<0.001
Prevalent stroke	234 (1.9)	23 (4.9)	211 (1.8)	<0.001
Prevalent diabetes	1354 (10.9)	172 (36.5)	1182 (9.9)	<0.001
eGFRcr (mL/min/1.73 m^2)	96.4 ± 15.6	90.8 ± 22.8	96.6 ± 15.2	<0.001

eGFRcys (mL/min/1.73 m^2)	91.0 ± 18.3	80.8 ± 23.6	91.4 ± 17.9	<0.001
eGFRcrcys (mL/min/1.73 m^2)	95.2 ± 17.0	86.7 ± 23.7	95.6 ± 16.6	<0.001
Cystatin C (mg/L)	0.9 [0.8- 1.0]	0.9 [0.8- 1.1]	0.9 [0.8- 1.0]	<0.001
B2M (mg/L)	1.8 [1.6- 2.1]	2.0 [1.8- 2.4]	1.8 [1.6- 2.1]	<0.001
FGF23 (pg/mL)	41.8 [33.9-51.6]	44.7 [35.5-57.0]	41.7 [33.8-51.4]	<0.001
PTH (pg/mL)	39.4 [31.2-49.5]	39.7 [29.9-49.6]	39.3 [31.2-49.5]	0.525
Calcium (mg/dL)	9.3 ± 0.4	9.4 ± 0.4	9.3 ± 0.4	0.064
Phosphorus (mg/dL)	3.5 ± 0.5	3.6 ± 0.6	3.5 ± 0.5	0.011

Note: Values for categorical variables are given as number (percentage); values for continuous variables are given as mean ± standard deviation or median [interquartile range].

Table 2 Hazard ratios and 95% confidence intervals for incident PAD, CLI according to clinical cutoffs of baseline eGFR using different markers

Markers	CKD Stage eGFR Clinical Cutoffs (mL/min/1.73 m^2)			
	>=90	60-<90	30-<60	<30
PAD				
Model 1				
eGFRcr	Referent	1.24(1.00,1.52)*	2.78(1.81,4.28)***	11.49(5.91,22.30)***
eGFRcys	Referent	1.31(1.06,1.63)*	2.53(1.85,3.47)***	11.24(6.45,19.59)***
eGFRcr-cys	Referent	1.54(1.25,1.89)***	3.75(2.62,5.37)***	13.14(7.13,24.20)***
Model 2				
eGFRcr	Referent	1.24(1.01,1.53)*	2.69(1.75,4.15)***	5.34(2.02,14.11)**
eGFRcys	Referent	1.31(1.06,1.62)*	2.52(1.84,3.45)***	7.05(3.53,14.08)***
eGFRcr-cys	Referent	1.54(1.25,1.90)***	3.73(2.59,5.35)***	7.47(3.24,17.24)***
CLI				
Model 1				
eGFRcr	Referent	0.79(0.54,1.16)	2.29(1.18,4.45)*	13.13(5.76,29.94)***
eGFRcys	Referent	1.10(0.77,1.59)	2.30(1.40,3.80)**	13.16(6.19,27.99)***
eGFRcr-cys	Referent	1.18(0.82,1.69)	3.09(1.73,5.52)***	12.99(5.68,29.68)***
Model 2				
eGFRcr	Referent	0.80(0.55,1.17)	2.21(1.14,4.30)*	3.57(0.98,12.96)
eGFRcys	Referent	1.09(0.75,1.56)	2.28(1.38,3.76)**	5.29(1.89,14.79)**
eGFRcr-cys	Referent	1.18(0.82,1.69)	2.97(1.65,5.34)***	3.56(1.00,12.68)*

Note:
*p<0.05, **p<0.01, ***p<0.001
Model 1 adjusted for age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke.
Model 2 adjusted for covariates in Model 1 plus FGF23, PTH, calcium and phosphorus.

Table 3 Hazard ratios and 95% confidence intervals for incident PAD, CLI according to quartiles of kidney function markers (1st quartile as referent)

Markers	Q1	Q2	Q3	Q4
		PAD		
		Model 1		
eGFRcr	Referent	0.72(0.52,0.99)*	0.96(0.71,1.29)	1.30(0.98,1.71)
eGFRcys	Referent	1.00(0.72,1.37)	1.12(0.82,1.53)	1.83(1.36,2.48)***
eGFRcr-cys	Referent	0.85(0.62,1.16)	1.05(0.78,1.43)	1.58(1.18,2.12)**
cystatin C	Referent	1.28(0.93,1.75)	1.07(0.76,1.49)	2.08(1.53,2.83)***
B2M	Referent	1.29(0.93,1.80)	1.75(1.27,2.40)**	2.85(2.10,3.88)***
		Model 2		
eGFRcr	Referent	0.71(0.52,0.97)*	0.93(0.69,1.26)	1.26(0.95,1.66)
eGFRcys	Referent	0.99(0.72,1.37)	1.13(0.83,1.54)	1.76(1.30,2.38)***
eGFRcr-cys	Referent	0.83(0.60,1.13)	1.04(0.77,1.41)	1.51(1.12,2.02)**
cystatin C	Referent	1.28(0.93,1.75)	1.08(0.77,1.50)	2.03(1.49,2.76)***
B2M	Referent	1.30(0.93,1.81)	1.76(1.28,2.42)**	2.75(2.02,3.74)***
		CLI		
		Model 1		
eGFRcr	Referent	0.58(0.34,1.00)*	1.12(0.70,1.81)	0.97(0.63,1.50)
eGFRcys	Referent	1.03(0.62,1.73)	1.25(0.76,2.05)	1.46(0.89,2.41)
eGFRcr-cys	Referent	1.06(0.65,1.72)	1.09(0.67,1.78)	1.39(0.87,2.23)
cystatin C	Referent	1.41(0.86,2.31)	1.18(0.70,1.98)	1.69(1.03,2.76)*
B2M	Referent	1.86(1.08,3.19)*	2.39(1.41,4.06)**	2.79(1.66,4.70)***
		Model 2		
eGFRcr	Referent	0.56(0.33,0.97)*	1.07(0.66,1.72)	0.88(0.57,1.37)
eGFRcys	Referent	1.04(0.62,1.75)	1.26(0.77,2.07)	1.34(0.81,2.20)
eGFRcr-cys	Referent	1.02(0.62,1.66)	1.07(0.65,1.74)	1.25(0.77,2.01)
cystatin C	Referent	1.43(0.87,2.35)	1.20(0.71,2.02)	1.57(0.95,2.58)
B2M	Referent	1.89(1.10,3.26)*	2.42(1.42,4.11)**	2.55(1.51,4.31)***

Note:
*p<0.05, **p<0.01, ***p<0.001
Model 1 adjusted for age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke.
Model 2 adjusted for covariates in Model 1 plus FGF23, PTH, calcium and phosphorus.
Quartiles for kidney function markers:
eGFRcr (mL/min/1.73 m^2): Q1: ≥ 105.64, Q2: 97.44-<105.64, Q3: 88.68-< 97.44, Q4: < 88.68
eGFRcys (mL/min/1.73 m^2): Q1: ≥ 104.88, Q2: 93.75-<104.88, Q3: 79.05-< 93.75, Q4: < 79.05
eGFRcr-cys (mL/min/1.73 m^2): Q1: ≥106.64, Q2: 96.69-<106.64, Q3: 85.19-< 96.69, Q4: < 85.19
cystatin C (mg/L): Q1: <0.76, Q2: 0.76-< 0.85, Q3: 0.85-< 0.97, Q4: ≥0.97
B2M (mg/L): Q1: <1.62, Q2: 1.62-< 1.84, Q3: 1.84-< 2.11, Q4: ≥2.11

Table 4 Hazard ratios and 95% confidence intervals for incident PAD, CLI according to quartiles of bone-mineral metabolism markers (1st quartile as referent)

Markers	Q1	Q2	Q3	Q4
		PAD		
		Model I		
FGF23	Referent	0.95(0.72,1.25)	1.09(0.83,1.43)	1.58(1.23,2.02)***
PTH	Referent	0.64(0.49,0.83)**	0.86(0.67,1.10)	0.80(0.62,1.03)
Calcium	Referent	1.07(0.81,1.40)	1.16(0.91,1.48)	1.22(0.94,1.57)
Phosphorus	Referent	1.09(0.84,1.40)	1.10(0.85,1.41)	1.62(1.23,2.13)**
		Model II		
FGF23	Referent	0.97(0.74,1.29)	1.02(0.78,1.33)	1.41(1.09,1.82)**
PTH	Referent	0.74(0.57,0.97)*	0.96(0.75,1.23)	0.92(0.71,1.20)
Calcium	Referent	0.95(0.72,1.25)	1.07(0.84,1.36)	0.95(0.73,1.23)
Phosphorus	Referent	1.16(0.90,1.50)	1.10(0.86,1.42)	1.52(1.15,2.01)**
		Model III		
FGF23	Referent	0.94(0.71,1.24)	0.96(0.73,1.26)	1.16(0.89,1.51)
PTH	Referent	0.73(0.56,0.95)*	0.97(0.76,1.25)	0.86(0.66,1.12)
Calcium	Referent	0.95(0.73,1.25)	1.04(0.82,1.33)	0.89(0.68,1.15)
Phosphorus	Referent	1.16(0.90,1.50)	1.11(0.86,1.43)	1.43(1.08,1.88)*
		CLI		
		Model I		
FGF23	Referent	0.70(0.44,1.11)	0.95(0.61,1.45)	1.31(0.88,1.95)
PTH	Referent	0.78(0.51,1.21)	0.90(0.60,1.37)	0.67(0.43,1.04)
Calcium	Referent	1.07(0.68,1.69)	1.00(0.66,1.52)	1.29(0.85,1.94)
Phosphorus	Referent	1.05(0.69,1.62)	1.01(0.66,1.54)	1.51(0.96,2.35)
		Model II		
FGF23	Referent	0.70(0.43,1.11)	0.84(0.55,1.30)	1.06(0.71,1.60)
PTH	Referent	0.88(0.57,1.36)	0.96(0.63,1.46)	0.73(0.46,1.14)
Calcium	Referent	0.92(0.58,1.46)	0.94(0.61,1.43)	0.94(0.61,1.45)
Phosphorus	Referent	1.18(0.76,1.83)	1.09(0.71,1.67)	1.59(1.01,2.51)*
		Model III		
FGF23	Referent	0.68(0.42,1.09)	0.81(0.53,1.25)	0.90(0.59,1.38)
PTH	Referent	0.86(0.55,1.33)	0.97(0.64,1.47)	0.67(0.42,1.05)
Calcium	Referent	0.94(0.59,1.49)	0.94(0.62,1.43)	0.93(0.61,1.42)
Phosphorus	Referent	1.19(0.77,1.84)	1.13(0.74,1.73)	1.54(0.98,2.42)

Note:
*p<0.05, **p<0.01, ***p<0.001
Model I adjusted for age, gender, race and ARIC visit center.
Model II adjusted for covariates in Model I plus education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke.
Model III adjusted for covariates in Model II plus eGFRcr-cys.

Quartiles for BMM markers:
FGF23 (pg/mL): Q1: <33.89, Q2: 33.89-<41.79, Q3: 41.79-< 51.57, Q4: ≥ 51.57
PTH (pg/mL): Q1: <31.20, Q2: 31.20-<39.38, Q3: 39.38-< 49.47, Q4: ≥49.47
Calcium (mg/dL): Q1: <9.2, Q2: 9.2-<9.4, Q3: 9.4-< 9.7, Q4: ≥9.7
Phosphorus (mg/dL): Q1: <3.3, Q2: 3.3-< 3.6, Q3: 3.6-< 4.0, Q4: ≥4.0

Supplementary tables

Table S 1 Spearman rank correlation coefficients between filtration markers and BMM markers at baseline

	eGFR cr	eGFR cys	eGFR cr-cys	cystatin C	B2M	FGF23	PTH	Ca	P
eGFRcr	1.000								
eGFRcys	0.519	1.000							
eGFRcr-cys	0.809	0.905	1.000						
cystatin C	-0.516	-0.968	-0.898	1.000					
B2M	-0.457	-0.772	-0.727	0.762	1.000				
FGF23	-0.163	-0.244	-0.238	0.249	0.237	1.000			
PTH	-0.003	-0.022	-0.007	-0.005	0.015	0.081	1.000		
Ca	-0.047	-0.123	-0.101	0.110	0.096	0.129	-0.091	1.000	
P	0.078	-0.042	0.019	-0.022	0.016	0.110	-0.134	0.201	1.000

Abbreviations:
B2M: beta-2 microglobulin, FGF23: fibroblast growth factor 23, PTH: parathyroid hormone,
Ca: calcium, P: phosphorus

Table S 2 Hazard ratios and 95% confidence intervals for incident PAD and CLI according to clinical cutoffs of baseline eGFR using different markers with adjustment for BMM markers in turn

Marker	Model	CKD Stage eGFR Clinical Cutoffs (mL/min/1.73 m^2)			
		>=90	60-<90	30-<60	<30
			PAD as Outcome		
eGFRcr	1	Referent	1.24(1.00,1.52)	2.78(1.81,4.28)	11.49(5.91,22.30)
	2.a	Referent	1.24(1.00,1.52)	2.78(1.81,4.28)	11.28(5.73,22.19)
	2.b	Referent	1.23(1.00,1.52)	2.77(1.80,4.26)	7.98(3.32,19.15)
	2.c	Referent	1.24(1.01,1.53)	2.79(1.81,4.30)	11.26(5.77,21.97)
	2.d	Referent	1.24(1.00,1.52)	2.70(1.75,4.17)	8.74(4.40,17.34)
	2	Referent	1.24(1.01,1.53)	2.69(1.75,4.15)	5.34(2.02,14.11)
eGFRcys	1	Referent	1.31(1.06,1.63)	2.53(1.85,3.47)	11.24(6.45,19.59)
	2.a	Referent	1.31(1.06,1.63)	2.53(1.85,3.47)	11.08(6.31,19.44)
	2.b	Referent	1.31(1.06,1.62)	2.51(1.83,3.44)	8.96(4.68,17.13)
	2.c	Referent	1.33(1.07,1.64)	2.55(1.86,3.50)	11.19(6.41,19.52)
	2.d	Referent	1.30(1.05,1.61)	2.52(1.84,3.45)	9.13(5.15,16.20)
	2	Referent	1.31(1.06,1.62)	2.52(1.84,3.45)	7.05(3.53,14.08)
eGFRcr-cys	1	Referent	1.54(1.25,1.89)	3.75(2.62,5.37)	13.14(7.13,24.20)
	2.a	Referent	1.54(1.25,1.89)	3.75(2.62,5.37)	12.94(6.96,24.08)
	2.b	Referent	1.53(1.24,1.89)	3.72(2.59,5.33)	10.14(4.71,21.85)
	2.c	Referent	1.55(1.26,1.91)	3.80(2.65,5.46)	12.83(6.94,23.72)
	2.d	Referent	1.53(1.24,1.88)	3.70(2.58,5.30)	10.39(5.53,19.52)
	2	Referent	1.54(1.25,1.90)	3.73(2.59,5.35)	7.47(3.24,17.24)
			CLI as Outcome		
eGFRcr	1	Referent	0.79(0.54,1.16)	2.29(1.18,4.45)	13.13(5.76,29.94)
	2.a	Referent	0.79(0.54,1.16)	2.30(1.18,4.45)	12.92(5.59,29.86)
	2.b	Referent	0.79(0.54,1.16)	2.27(1.17,4.39)	6.22(2.03,19.05)
	2.c	Referent	0.80(0.54,1.17)	2.29(1.18,4.43)	12.80(5.53,29.64)
	2.d	Referent	0.80(0.54,1.17)	2.25(1.16,4.38)	9.48(4.00,22.49)
	2	Referent	0.80(0.55,1.17)	2.21(1.14,4.30)	3.57(0.98,12.96)
eGFRcys	1	Referent	1.10(0.77,1.59)	2.30(1.40,3.80)	13.16(6.19,27.99)
	2.a	Referent	1.10(0.76,1.58)	2.30(1.40,3.79)	12.97(6.05,27.79)
	2.b	Referent	1.08(0.75,1.56)	2.24(1.36,3.69)	7.74(3.07,19.56)
	2.c	Referent	1.11(0.77,1.60)	2.31(1.40,3.81)	12.89(6.04,27.52)
	2.d	Referent	1.10(0.76,1.58)	2.34(1.42,3.86)	10.08(4.60,22.10)
	2	Referent	1.09(0.75,1.56)	2.28(1.38,3.76)	5.29(1.89,14.79)

eGFRcr-cys	1	Referent	1.18(0.82,1.69)	3.09(1.73,5.52)	12.99(5.68,29.68)
	2.a	Referent	1.18(0.82,1.69)	3.09(1.73,5.53)	12.75(5.51,29.46)
	2.b	Referent	1.16(0.81,1.67)	3.01(1.68,5.39)	6.20(2.07,18.57)
	2.c	Referent	1.19(0.83,1.70)	3.11(1.74,5.57)	12.42(5.37,28.72)
	2.d	Referent	1.19(0.83,1.70)	3.02(1.68,5.43)	9.70(4.11,22.88)
	2	Referent	1.18(0.82,1.69)	2.97(1.65,5.34)	3.56(1.00,12.68)

Note:
Model 1 adjusted for age, gender, race and ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke.
Model 2.a adjusted for covariates in Model 1 plus FGF23.
Model 2.b adjusted for covariates in Model 1 plus PTH.
Model 2.c adjusted for covariates in Model 1 plus calcium.
Model 2.d adjusted for covariates in Model 1 plus phosphorus.
Model 2 adjusted for covariates in Model 1 plus FGF23, PTH, calcium and phosphorus.

Table S 3 Subgroup analyses on the association between incident PAD and every 15-unit decrease in eGFRs

Outcome	Marker	Demographical factor	Level	<105 Slope per 15-unit [a] decrease	p-value
PAD	eGFRcr	Age			0.033
			≥65	1.36 (1.23,1.51)	
			<65	1.45 (1.32,1.58)	
PAD	eGFRcr	Sex			0.833
			Male	1.42 (1.26,1.61)	
			Female	1.36 (1.21,1.54)	
PAD	eGFRcr	Race			0.708
			Black	1.44 (1.25,1.65)	
			White	1.36 (1.21,1.54)	
PAD	eGFRcr	Ever smoking			0.000
			Yes	1.32 (1.20,1.45)	
			No	1.52 (1.37,1.67)	
PAD	eGFRcr	Diabetes			0.030
			Yes	1.32 (1.17,1.49)	
			No	1.46 (1.30,1.65)	
PAD	eGFRcr	Hypertension			0.000
			Yes	1.34 (1.22,1.47)	
			No	1.49 (1.35,1.63)	
PAD	eGFRcr	Prevalent CVD			0.187
			Yes	1.26 (1.07,1.49)	
			No	1.43 (1.29,1.59)	
PAD	eGFRcys	Age			0.099
			≥65	1.36 (1.23,1.49)	
			<65	1.43 (1.32,1.55)	
PAD	eGFRcys	Sex			0.961
			Male	1.38 (1.23,1.53)	
			Female	1.38 (1.24,1.54)	

PAD	eGFRcys	Race			0.253
			Black	1.38 (1.22,1.57)	
			White	1.37 (1.24,1.52)	
PAD	eGFRcys	Ever smoking			0.000
			Yes	1.37 (1.26,1.49)	
			No	1.55 (1.42,1.70)	
PAD	eGFRcys	Diabetes			0.023
			Yes	1.29 (1.15,1.45)	
			No	1.45 (1.31,1.61)	
PAD	eGFRcys	Hypertension			0.000
			Yes	1.33 (1.22,1.45)	
			No	1.47 (1.35,1.60)	
PAD	eGFRcys	Prevalent CVD			0.238
			Yes	1.25 (1.07,1.46)	
			No	1.43 (1.30,1.57)	
PAD	eGFRcr-cys	Age			0.044
			≥65	1.43 (1.29,1.58)	
			<65	1.51 (1.39,1.65)	
PAD	eGFRcr-cys	Sex			0.976
			Male	1.47 (1.31,1.65)	
			Female	1.45 (1.29,1.63)	
PAD	eGFRcr-cys	Race			0.376
			Black	1.48 (1.30,1.69)	
			White	1.43 (1.28,1.60)	
PAD	eGFRcr-cys	Ever smoking			0.000
			Yes	1.42 (1.30,1.55)	
			No	1.62 (1.48,1.78)	

PAD	eGFRcr-cys	Diabetes			0.016
			Yes	1.36 (1.21,1.53)	
			No	1.54 (1.38,1.73)	
PAD	eGFRcr-cys	Hypertension			0.000
			Yes	1.41 (1.29,1.54)	
			No	1.55 (1.42,1.70)	
PAD	eGFRcr-cys	Prevalent CVD			0.190
			Yes	1.29 (1.09,1.52)	
			No	1.52 (1.38,1.68)	

Note:
a. The unit for eGFRs is mL/min/1.73 m^2.
HRs were calculated from linear combinations of coefficients in the model with interaction terms based on model 2, and p-value was obtained from likelihood ratio test comparing models with and without the interaction term.
Specifically,
(1) when stratifying by age, covariates in the model included main effects of the binary age category and both of the two slopes as well as their interactions, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(2) when stratifying by gender, covariates in the model included main effects of gender and both of the two slopes as well as their interactions, age, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(3) when stratifying by race, covariates in the model included main effects of race and both of the two slopes as well as their interactions, age, gender, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(4) when stratifying by ever smoking, covariates in the model included main effects of binary smoking category (ever vs. never smoking) and both of the two slopes as well as their interactions, age, gender, race, ARIC visit center, education level, BMI, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(5) when stratifying by diabetes status, covariates in the model included main effects of binary indicator for diabetes and both of the two slopes as well as their interactions, age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(6) when stratifying by hypertension status, covariates in the model included main effects of hypertension and each of the two slopes as well as their interactions, age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke, plus FGF23, PTH, calcium and phosphorus;
(7) when stratifying by prevalent CVD, covariates in the model included main effects of prevalent CVD and each of the two slopes as well as their interactions, age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, plus FGF23, PTH, calcium and phosphorus.

Table S 4 Hazard ratios and 95% confidence intervals for incident PAD, CLI according to quartiles of kidney function markers (2nd quartile as referent)

Markers	Q1	Q2	Q3	Q4
		PAD		
		Model 1		
eGFRcr	1.40(1.03,1.89)*	Referent	1.27(0.95,1.69)	1.82(1.40,2.38)***
eGFRcys	1.03(0.75,1.43)	Referent	1.17(0.88,1.57)	1.97(1.51,2.59)***
eGFRcr-cys	1.12(0.82,1.54)	Referent	1.28(0.96,1.71)	1.92(1.46,2.51)***
cystatin C	0.71(0.52,0.97)*	Referent	0.82(0.62,1.08)	1.66(1.29,2.14)***
B2M	0.83(0.60,1.15)	Referent	1.42(1.06,1.89)*	2.29(1.74,3.01)***
		Model 2		
eGFRcr	1.44(1.05,1.99)*	Referent	1.40(1.03,1.89)*	1.88(1.41,2.51)***
eGFRcys	1.03(0.74,1.43)	Referent	1.19(0.89,1.60)	1.88(1.43,2.48)***
eGFRcr-cys	1.15(0.84,1.59)	Referent	1.31(0.98,1.75)	1.85(1.40,2.43)***
cystatin C	0.70(0.51,0.97)*	Referent	0.83(0.62,1.10)	1.59(1.23,2.06)***
B2M	0.84(0.60,1.17)	Referent	1.44(1.08,1.93)*	2.21(1.68,2.92)***
		CLI		
		Model 1		
eGFRcr	1.72(1.00,2.95)*	Referent	1.93(1.13,3.29)*	1.67(1.00,2.80)
eGFRcys	0.97(0.58,1.62)	Referent	1.20(0.76,1.91)	1.42(0.91,2.21)
eGFRcr-cys	0.95(0.58,1.54)	Referent	1.03(0.65,1.65)	1.32(0.85,2.04)
cystatin C	0.71(0.43,1.16)	Referent	0.83(0.53,1.31)	1.20(0.79,1.80)
B2M	0.54(0.31,0.93)*	Referent	1.29(0.82,2.01)	1.51(0.98,2.32)
		Model 2		
eGFRcr	1.77(1.03,3.04)*	Referent	1.89(1.11,3.23)*	1.56(0.93,2.63)
eGFRcys	0.96(0.57,1.61)	Referent	1.21(0.76,1.92)	1.28(0.82,2.02)
eGFRcr-cys	0.99(0.60,1.61)	Referent	1.05(0.66,1.68)	1.23(0.79,1.91)
cystatin C	0.70(0.43,1.15)	Referent	0.84(0.53,1.32)	1.09(0.72,1.66)
B2M	0.53(0.31,0.91)*	Referent	1.28(0.82,2.00)	1.35(0.87,2.09)

Note:
*p<0.05, **p<0.01, ***p<0.001
Model 1 adjusted for age, gender, race, ARIC visit center, education level, BMI, smoking status, alcohol drinking status, LDL level, HDL level, systolic blood pressure, anti-hypertensive medication, lowering-cholesterol medication, diabetes, prevalent coronary heart disease and prevalent stroke.
Model 2 adjusted for covariates in Model 1 plus FGF23, PTH, calcium and phosphorus.
Quartiles for kidney function markers:
eGFRcr (mL/min/1.73 m^2): Q1: ≥ 105.64, Q2: 97.44-<105.64, Q3: 88.68-< 97.44, Q4: < 88.68
eGFRcys (mL/min/1.73 m^2): Q1: ≥ 104.88, Q2: 93.75-<104.88, Q3: 79.05-< 93.75, Q4: < 79.05
eGFRcr-cys (mL/min/1.73 m^2): Q1: ≥106.64, Q2: 96.69-<106.64, Q3: 85.19-< 96.69, Q4: < 85.19
cystatin C (mg/L): Q1: <0.76, Q2: 0.76-< 0.85, Q3: 0.85-< 0.97, Q4: ≥0.97
B2M (mg/L): Q1: <1.62, Q2: 1.62-< 1.84, Q3: 1.84-< 2.11, Q4: ≥2.11

Table S 5 Subgroup analyses on the association with incident PAD and CLI across quartiles of cystatin C and beta-2 microglobulin (1st quartile as referent)

Marker	Demographical factor	Level	Q1	Q2	Q3	Q4	p-value
				PAD as Outcome			
Cystatin C	Age (years)	<65	Referent	1.28(0.92-1.78)	1.01(0.71-1.44)	2.18(1.59-2.99)	0.022
		≥65	Referent	2.67(0.78-9.19)	3.22(0.96-10.85)	4.10(1.27-13.24)	
	Sex	Female	Referent	1.24(0.80-1.92)	1.09(0.69-1.73)	2.02(1.35-3.03)	0.993
		Male	Referent	1.30(0.82-2.07)	1.06(0.66-1.72)	1.98(1.27-3.08)	
	Race	White	Referent	1.54(1.00-2.38)	1.37(0.88-2.14)	2.52(1.66-3.83)	0.318
		Black	Referent	1.08(0.67-1.75)	0.78(0.45-1.34)	1.51(0.97-2.36)	
	Ever smoking	No	Referent	1.18(0.65-2.13)	1.16(0.63-2.11)	2.33(1.36-3.98)	0.000
		Yes	Referent	1.46(1.01-2.12)	1.22(0.82-1.80)	2.34(1.64-3.34)	
	Diabetes	No	Referent	1.48(0.97-2.27)	1.26(0.81-1.96)	2.57(1.71-3.87)	0.220
		Yes	Referent	1.10(0.68-1.78)	0.91(0.55-1.51)	1.44(0.93-2.22)	
	Hypertension	No	Referent	1.21(0.78-1.86)	1.06(0.67-1.67)	1.76(1.15-2.71)	0.000
		Yes	Referent	1.39(0.88-2.20)	1.13(0.71-1.82)	2.23(1.47-3.39)	
	Prevalent CVD	No	Referent	1.30(0.93-1.82)	0.94(0.65-1.36)	2.09(1.50-2.90)	0.077
		Yes	Referent	1.18(0.47-2.94)	1.78(0.76-4.17)	1.86(0.84-4.12)	
B2M	Age (years)	<65	Referent	1.21(0.86-1.71)	1.65(1.19-2.30)	2.77(2.02-3.79)	0.015
		≥65	Referent	5.38(1.19-24.25)	7.67(1.80-32.70)	10.21(2.45-42.55)	
	Sex	Female	Referent	1.32(0.80-2.18)	1.82(1.14-2.89)	2.55(1.64-3.96)	0.869
		Male	Referent	1.25(0.81-1.94)	1.67(1.09-2.54)	2.81(1.88-4.20)	
	Race	White	Referent	1.34(0.85-2.10)	1.99(1.31-3.02)	3.01(2.00-4.53)	0.599
		Black	Referent	1.29(0.78-2.12)	1.35(0.79-2.31)	2.33(1.48-3.66)	

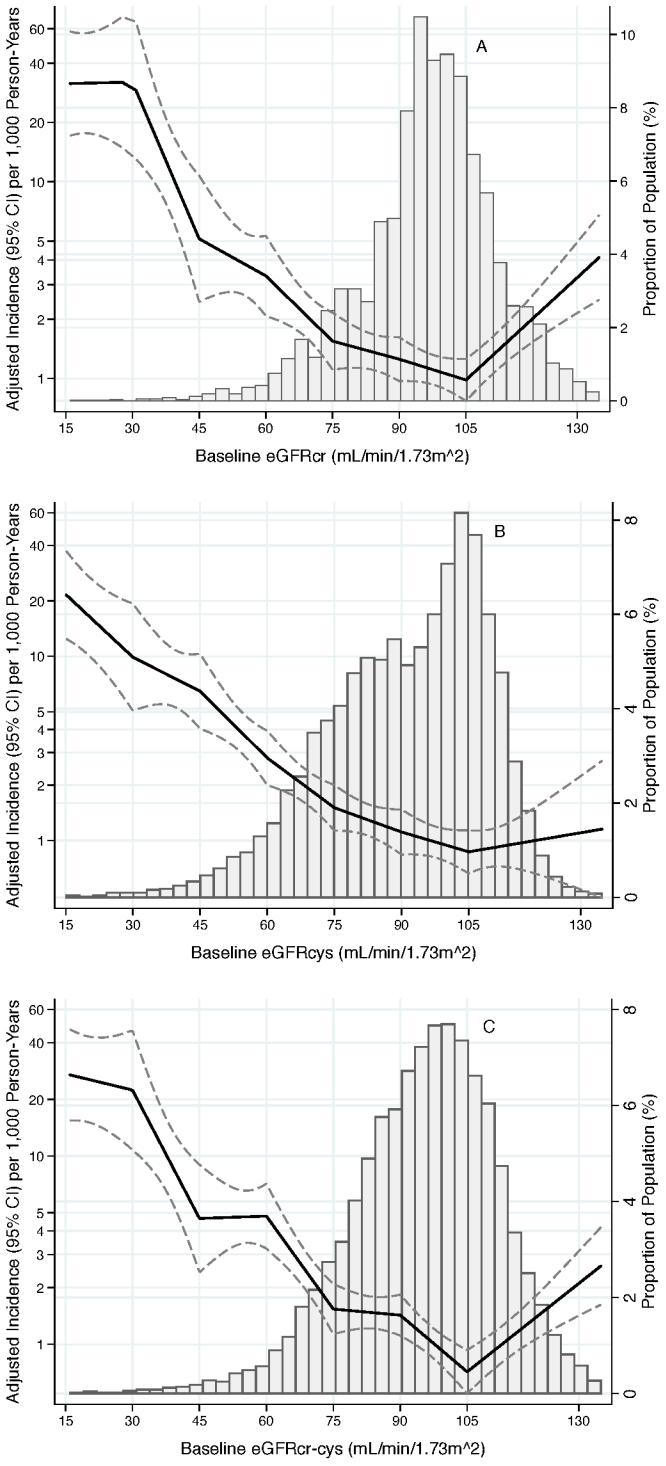
	Ever smoking	No	Referent	0.72(0.35-1.46)	1.48(0.82-2.67)	2.56(1.50-4.35)	0.000
		Yes	Referent	1.56(1.07-2.29)	1.87(1.29-2.71)	2.74(1.92-3.92)	
	Diabetes	No	Referent	1.33(0.85-2.08)	2.04(1.34-3.09)	3.33(2.22-4.99)	0.164
		Yes	Referent	1.32(0.80-2.18)	1.39(0.85-2.28)	2.00(1.29-3.10)	
	Hypertension	No	Referent	1.34(0.85-2.13)	1.80(1.16-2.80)	2.63(1.70-4.06)	0.000
		Yes	Referent	1.20(0.75-1.93)	1.64(1.05-2.55)	2.70(1.79-4.06)	
	Prevalent CVD	No	Referent	1.21(0.84-1.73)	1.70(1.21-2.39)	2.78(2.00-3.87)	0.550
		Yes	Referent	1.73(0.73-4.07)	1.94(0.85-4.42)	2.53(1.19-5.37)	
				CLI as Outcome			
	Age	<65	Referent	1.61(0.95-2.73)	1.24(0.70-2.19)	1.74(1.03-2.95)	0.196
		≥65	Referent	1.13(0.28-4.56)	1.61(0.42-6.13)	1.99(0.57-6.92)	
	Sex	Female	Referent	1.28(0.66-2.51)	1.22(0.62-2.41)	1.65(0.89-3.06)	0.912
		Male	Referent	1.57(0.74-3.30)	1.17(0.53-2.60)	1.52(0.72-3.20)	
Cystatin C	Race	White	Referent	2.16(0.93-5.02)	1.62(0.68-3.87)	2.02(0.87-4.68)	0.590
		Black	Referent	1.07(0.56-2.06)	1.03(0.52-2.05)	1.48(0.81-2.72)	
	Ever smoking	No	Referent	0.99(0.44-2.22)	0.80(0.34-1.88)	1.71(0.82-3.54)	0.081
		Yes	Referent	1.89(1.01-3.54)	1.67(0.87-3.20)	1.79(0.96-3.34)	
	Diabetes	No	Referent	1.51(0.68-3.37)	1.14(0.49-2.68)	1.97(0.90-4.30)	0.699
		Yes	Referent	1.41(0.75-2.65)	1.28(0.67-2.43)	1.38(0.76-2.52)	
	Hypertension	No	Referent	1.30(0.57-2.94)	1.24(0.53-2.92)	1.51(0.66-3.47)	0.000
		Yes	Referent	1.53(0.83-2.82)	1.19(0.63-2.27)	1.62(0.90-2.92)	
	Prevalent CVD	No	Referent	1.55(0.91-2.63)	1.08(0.61-1.92)	1.76(1.03-3.01)	0.201
		Yes	Referent	0.81(0.20-3.32)	1.73(0.52-5.77)	1.06(0.35-3.25)	
	Age (years)	<65	Referent	1.69(0.95-2.99)	2.20(1.25-3.86)	2.70(1.57-4.65)	0.105
		≥65	Referent	7.08(0.86-58.26)	9.42(1.21-73.12)	7.20(0.93-55.79)	

	Sex	Female	Referent	2.13(0.97-4.68)	2.11(0.97-4.57)	2.85(1.38-5.85)	0.600
		Male	Referent	1.66(0.80-3.48)	2.68(1.32-5.41)	2.22(1.08-4.56)	
B2M							
	Race	White	Referent	2.86(1.07-7.70)	3.65(1.41-9.47)	3.36(1.29-8.77)	0.562
		Black	Referent	1.48(0.74-2.95)	1.82(0.90-3.66)	2.41(1.29-4.52)	
	Ever smoking	No	Referent	0.39(0.13-1.23)	1.30(0.59-2.88)	1.91(0.94-3.90)	0.001
		Yes	Referent	3.79(1.85-7.79)	3.69(1.79-7.63)	3.25(1.57-6.72)	
	Diabetes	No	Referent	1.26(0.51-3.11)	2.24(0.98-5.09)	2.97(1.32-6.66)	0.184
		Yes	Referent	2.46(1.26-4.83)	2.52(1.28-4.96)	2.26(1.18-4.33)	
	Hypertension	No	Referent	2.27(0.96-5.39)	1.85(0.75-4.57)	2.73(1.14-6.54)	0.000
		Yes	Referent	1.62(0.81-3.23)	2.63(1.38-5.01)	2.50(1.33-4.67)	
	Prevalent CVD	No	Referent	1.70(0.95-3.03)	2.21(1.26-3.87)	2.59(1.49-4.53)	0.581
		Yes	Referent	3.71(0.74-18.68)	4.28(0.89-20.71)	3.11(0.71-13.66)	

Note:
HRs were calculated from linear combinations of coefficients in the model with interaction terms based on model 2, and p-value was obtained from likelihood ratio test comparing models with and without the interaction term.
Detailed descriptions on covariates included in the model for different stratification variables can be found under Table S3.
Quartiles for cystatin C and B2M:
Cystatin C (mg/L): Q1: <0.76, Q2: 0.76-< 0.85, Q3: 0.85-< 0.97, Q4:≥0.97
B2M (mg/L): Q1: <1.62, Q2: 1.62-< 1.84, Q3: 1.84-< 2.11, Q4: ≥2.11
Abbreviation:
CVD: cardiovascular disease

FIGURES

Figure 1 Age-, gender-, and race-adjusted incident rate of PAD according to eGFRcr (A), eGFRcys (B), and eGFRcr-cys (C) (with knots at 30, 45, 60, 75, 90 and 105 ml/min/1.73m^2



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EDUCATION
Master of Science Expected May 2016
Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
Major: Epidemiology, with concentration in cardiovascular disease
Bachelor of Medicine July 2014
Fudan University Shanghai Medical College, Shanghai, China
Major: Preventive Medicine
Donghua University, Shanghai, China Sept. 2008- July 2009
Major: Electrical Engineering

MANUSCRIPT & PUBLICATION
Yang C., Kwak L., Ballew S., et al. *Kidney function, bone-mineral metabolism markers, and peripheral artery disease: the Atherosclerosis Risk in Communities (ARIC) Study*. Manuscript in preparation
Xu F., **Yang C.**, Chai W.H., et al. (2012) Childhood Atopic Dermatitis and Household Environmental Risk Factors- A Cross-sectional Study in 4784 children in Jiading District, Shanghai, *Journal of Environment and Health, 2012, 29(6)*, 23-26.

CONFERENCE PRESENTATION
American Heart Association Epidemiology and Prevention, Lifestyle and Cardiometabolic Health 2016 Scientific Sessions (AHA-EPI), Phoenix, AZ Mar 2 2016
Moderated presentation of abstract: *Kidney function, bone-mineral metabolism markers, and peripheral artery disease: the Atherosclerosis Risk in Communities (ARIC) Study*.

RESEARCH & TEACHING EXPERIENCE
Teaching Assistant Mar 2016-
for *Stata Programming*
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Instructors: Profs. Allan Massie and Dorry Segev
- Instructed students to master programming skills in data management and automated table-making using Stata
- Graded homework

Research Assistant Jul 2015-
Department of Epidemiology,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Supervisor: Prof. Kunihiro Matsushita
- Extracted literature
- Summarized diseases' revised Current Procedural Terminology (CPT) codes
- Analyzed ARIC study data and drafted a manuscript

Teaching Assistant Sept 2015- Mar 2016
for *Statistical Methods in Public Health I to III* series
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Instructors: Profs. Marie Diener-West and Karen Bandeen-Roche
- Instructed students to solve homework problems
- Graded homework and exams

Research Assistant Jun 2015- Aug 2015
Epidemiological Research Group on Transplantation (ERGOT),
Johns Hopkins University School of Medicine, Baltimore, MD.
Supervisor: Prof. Allan Massie
- Cleaned a large ongoing REDCap dataset with more than 6,000 variables
- Conducted validation check and reshaped wide dataset into long format

Research Assistant Apr 2011- Jun 2014
Department of Environmental Health, School of Public Health,
Fudan University, Shanghai, China.
Supervisor: Prof. Zhuohui Zhao
- Collaborated on data collection and validation on a community-based cross-sectional survey in over 10,000 children aged 3-12 years old in three communities in Shanghai, China
- Analyzed study data and published the results in a Chinese core journal

AWARDS & HONORS
- Master's Tuition Scholarship from Johns Hopkins Bloomberg School of Public Health 2015
- Renmin Scholarship from Fudan University (for each academic year from 2011 through 2014) (Top 20%)
- Honor of Outstanding Student from Fudan University 2012 (Top 5%)
- Honor of Outstanding Youth Volunteer from Fudan University 2012 (Top 1%)
- Model Student Scholarship from Donghua University 2009 (Top 20%)

PROFESSIONAL TRAININGS
Program Intern, Jing'an District CDC, Shanghai, China Feb 2014- Apr 2014
- Assisted completing Jing'an District 2013-2014 Annual Vital Statistics Report

Medical Intern, Shanghai Fifth People's Hospital, Shanghai, China Jan 2013- Jul 2013
- Rotated in 12 clinical departments, including cardiology, nephrology and emergency room
- Supervised clinical care for hospitalized patients

PERSONAL DEVELOPMENT
Academic Performance
Graduate:
- Johns Hopkins University, GPA: 3.94/ 4

Undergraduate:

- Fudan University, GPA: 3.31/ 4, major GPA: 3.52/ 4 (rank 4/40)
- Donghua University, GPA: 3.2/ 4 (rank 41/396)

Statistical software competency and computer skills

- Experienced in Stata, R, SAS, Python and SPSS
- Certificate: SAS Certified Base Programmer for SAS 9

Language Skills

Fluent in English and Chinese